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Four antigens were found in the CB-1A complex mixture of low molecular weight protein and polysaccharidic proteins considered to be the principal allergens of castor beans. The principal antigen and a principal minor antigen were both allergenic.

THE CHEMISTRY OF ALLERGENS XVIII. An Analysis of CB-1A from Castor Beans*

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CB-1A is a complex mixture of low-molecular weight proteins and polysaccharidic proteins which contains the principal allergen(s) of castor beans, immunologically distinct from other allergens and antigens present in castor bean meal.[1,2]

The object of this paper is to describe a cellulose acetate electrophoretic fractionation of CB-1A, a spectrophotometric method of analysis of the CB-1A. Ponceau S complex, gel diffusion analysis of fractions and interpretation of the results obtained.

Apparatus and Materials**

CB-1A. CB-1A was isolated from defatted castor beans as previously described (3,4).

Electrophoresis Apparatus. The Shandon Electrophoresis apparatus† with 5 x 14 cm strips of Oxoid cellulose acetate† was used.

Buffer Solution. Barbitone acetate buffer,† 8.8 g per liter, pH 8.6, containing 0.025 per cent thymol, was used for the electrophoresis.

Agar. Ionagar, No. 2,† 0.5 per cent in buffered saline, pH 7.0, containing 0.01 per cent merthiolate, was used for the gel diffusion tests.

CB-1A Rabbit Antiserum. Rabbits were immunized to CB-1A by a series of inoculations with CB-1A in Freund's complete adjuvant as described for the preparation of CB-13E antiserum.[5]

Ponceau S[3]

Methods

Cellulose Acetate Electrophoresis. The CB-1A solution, at the concentration shown in Figure 1, was placed in the center of the strip, 3 cm from the anode. Electrophoresis was conducted for six hours at approximately 25° C at a constant current of 2 milliamperes per strip. Voltage was approximately 200 at the start and 130 at the end of the run. Strips were dried and stained with alcholic Ponceau S as previously described.[1]

Separation and Extraction of Fractions. The separated bands were cut from strips, Figure 1, and corresponding bands were combined and extracted with 0.07 N ammonium hydroxide. The fractions were recovered at once by lyophilization of the extracts.

*For the previous paper in this series see reference No. 2.

**Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

†Consolidated Laboratories, Chicago Heights, Illinois.

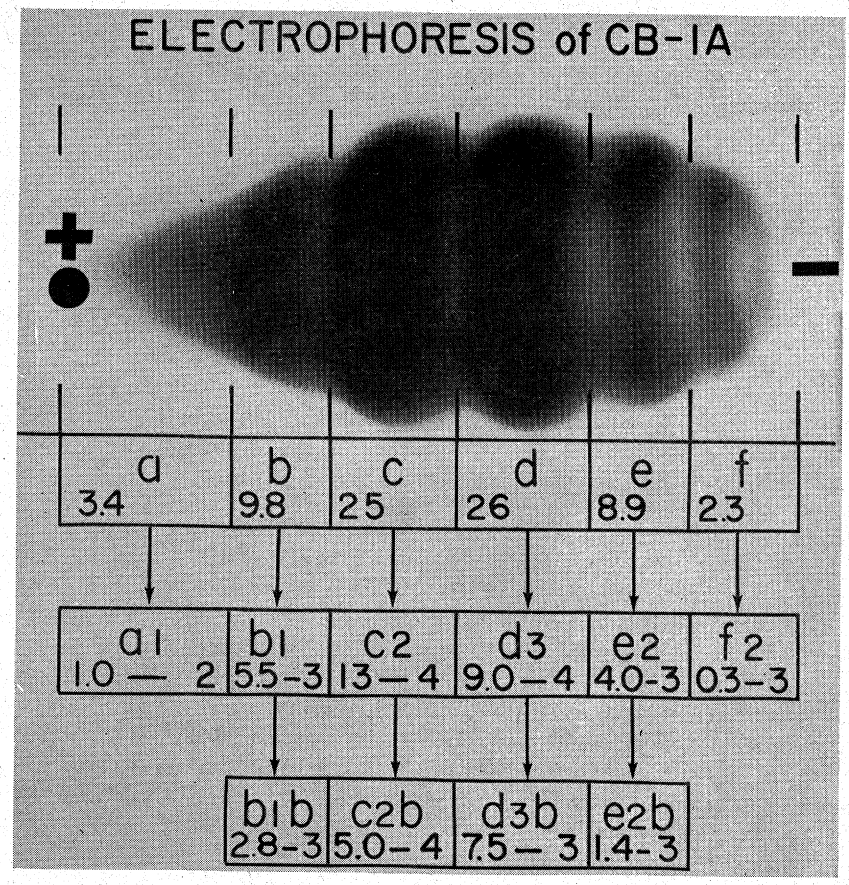

Fig. 1. Cellulose Acetate Electrophoretic Fractionation of CB-1A and of CB-1A • Ponceau S Sub-fractions. First fractionation: 143 mg of CB-1A in 25 per cent solution, 57 runs, 10 μl per run. Second fractionation: 10 μl of 5 per cent solutions was used (except 2.5 per cent solutions were used for fractions a and f) per run. Third fractionation: 10 μl of five per cent solutions per run. The number on the left in each box is the yield of fraction in mg. The numeral on the right is the number of visible bands on the strip. The heaviest band was always used for refractionation or for the final fraction in each case.

Analysis of Fractions. The CB-1A content of the ammonium hydroxide extracts was estimated spectrophotometrically by the absorbance at 520 mμ, the wave length of maximum absorption of Ponceau S. A standard curve was prepared with dried Ponceau S dissolved in 0.07 N ammonium hydroxide solution.

CB-1A•Ponceau S. To 500 mg of CB-1A in 25 ml of water was added 25 ml of one per cent Ponceau S in five per cent acetic acid (excess). The suspension stood at room temperature overnight. The supernatant solution was decanted and discarded. The gummy, red solid was washed seven times with 20-ml portions of five per cent acetic acid, then washed three times with 20-ml volumes of a solution containing 67 per cent ethanol and 33 per cent of five per cent aqueous acetic acid and finally washed twice with 50-ml volumes of 50 per cent ethanol. This treatment re-

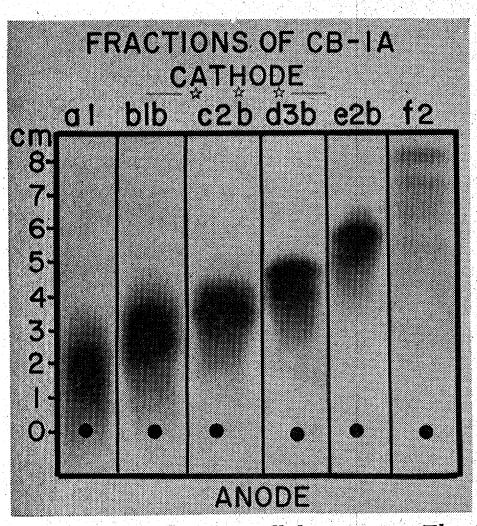

Fig. 2. Comparative Cellulose Acetate Electrophoretic Characterization of Final CB-1A • Ponceau S Fractions of CB-1A. 10-μl samples of solutions which contained 5 mg CB-1A per μl in Barbitone were used per run.

moved excess Ponceau S. The solid was dried in a vacuum over calcium chloride. The yield was 519 mg. Spectrophotometric analysis of this CB-1A• Ponceau S complex, dissolved in 0.07 N ammonium hydroxide, showed that it contained 56.5 per cent CB-1A. This value was used to calculate the CB-1A content of the CB-1A•Ponceau S fractions.

Refractionation of CB-1A•Ponceau S Fractions. The initial fractions obtained from the separated bands, Figure 1, were each refractionated by electrophoresis. During electrophoresis Ponceau S migrated rapidly toward the anode into the electrolyte chamber while the CB-1A migrated on the strip toward the cathode. The refractionations were repeated until the quantity of fraction became too small to continue.

Gel Diffusion Technique. The Ouchterlony technique [1,6] was used to show the specificity relationships of the final fractions. The Ponceau S in the fractions did not interfere with the gel diffusion analysis.

Results

Results of the fractionation of CB-1A and of the CB-1A • Ponceau S subfractions are shown in Figure 1.

Electrophoretic characterization of each of the final CB-1A•Ponceau S fractions is shown in Figure 2.

The antigenic specificity relationships of the final CB-1A•Ponceau S fractions are shown in Figure 3.

Discussion

Fractions a, b, c and d, whose subfractions contained the principal common antigenic specificity, accounted for about 85 per cent of the total solids from the first electrophoretic fractionation of CB-1A. Fractions e2b and f2 contained the principal minor antigenic specificities.

Electrophoretic characterization of

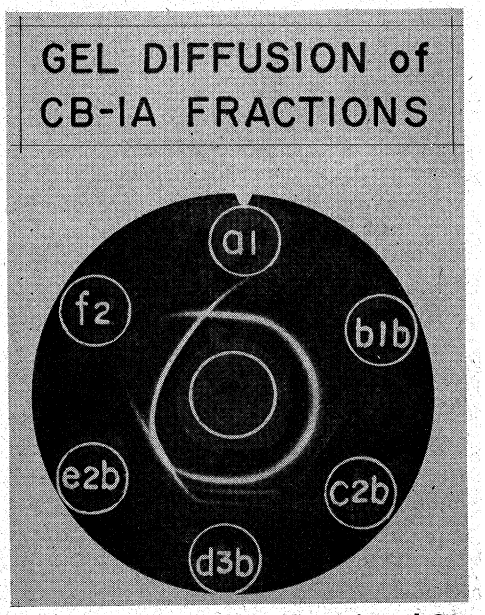

Fig. 3. Gel Diffusion Analysis of Final CB-1A • Ponceau S Fractions of CB-1A. Center well: CB-1A rabbit antiserum, 0.15 ml. Outer wells: 0.1 ml of solution containng 0.1 mg of indicated fraction of CB-1A • Ponceau S per ml in buffered saline, pH, 7.0. The antiserum diffused 24 hours before one filling with antigen solution. Photograph was taken after diffusion at 24° C for five days.

the final fractions, Figure 2, showed that Fraction d3b was essentially separated from Fraction a1. Nevertheless the gel diffusion analysis shown in Figure 3 indicates that fractions d3b and a1, as well as the intermediate fractions, b1b and c2b, contained a common specificity. Fraction e2b, shown in Figure 3, contained both the principal and minor specificity and f2 contained only the principal minor specificity. Two other specificities are present in trace amounts, one in Fractions e2b and d3b, and one in Fractions d3b, c2b and b1b.

It is apparent that the formation of a discrete band on cellulose acetate electrophoresis of such a heterogeneous mixture of closely related proteins does not indicate either a distinct antigenic specificity or a distinct chemical entity. Thus, Fraction d, Figure 1, for example, separated into four bands on re-electrophoresis and the single principal band d3 separated into three bands.

These results reaffirm the conclusions of our previous studies that chemically distinct principal components of CB-1A contain an identical antigenic specificity and in addition reveal other minor components with distinct specificities. Other examples of chemically different components exhibiting identical antigenic specificities have been observed in other systems, such as ribonuclease A and B, and B-lactoglobulin A and B.

Fractions a1 and f2, which represented the extremes of separation, were tested cutaneously on a castor bean sensitive person (Type II (4)) who had reagins for CB-1A. Both fractions gave 2+ reactions with pseudopods at 1:500,000. Fraction a1, the principal antigen, appeared to be the more potent, however, because it gave an equally strong reaction at 1:2,000,000 whereas f2 gave a ± reaction at this dilution. These results show that the two distinct antigens were also allergenic.

Summary

Four antigens were demonstrated in the CB-1A • Ponceau S complex by gel diffusion analysis of the electrophoretic fractions. The principal antigen was estimated as 85 per cent of the total complex from the first fractionation of CB-1A. The principal antigen and the principal minor antigen were essentially completely separated and both were allergenic.

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